PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20-01-2005
Applicant's or agent's file reference 2031002PC/ko	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI 2004/000540	15.09.2004	15.09.2003

International Patent Classification (IPC) or both national classification and IPC
C12N 15/70, C12N 15/61

Applicant
Fit Biotech OYJ PLC et al

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further opinions, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Form PCT/ISA/237 (cover sheet) (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/FI 2004/000540

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language, _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☐ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☐ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/FI 2004/000540

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-7, 9-24	YES
	Claims	8	NO
Inventive step (IS)	Claims	1-7, 16-24	YES
	Claims	8-15	NO
Industrial applicability (IA)	Claims	1-24	YES
	Claims		NO

2. Citations and explanations:

The present application relates to an antibiotic resistance-free selection system, which is based on the use of an araD gene, a complementary sequence thereof or a catalytically active fragment thereof as a selection marker carried on a plasmid which is inserted in a bacterial strain deficient of the araD gene. Further described, are vectors comprising the araD gene, E. coli strains deficient of the araD gene and a method of selecting the cells transformed with a plasmid containing the araD gene.

The independent claims 1, 8, 13-22, are not considered to be sufficiently defined since they refer merely to the gene name "araD". In order to render the subject-matter of the claims clear and defined (PCT Art 6), the gene should be defined in the claims by reference to the sequence identity number(s) of the nucleotide sequence(s) of the gene, as disclosed in the sequence listing of the application.

Regarding the use of a vector carrying a complementary sequence of the araD gene (included in the subject-matter of claims 1, 8 and 22) it seems unlikely that the protein which is encoded by the complementary sequence of the araD gene would have the same functions as the protein encoded by the araD gene (unless the araD DNA sequence were a palindrome). Consequently, a selection system comprising a vector carrying the complementary sequence of the araD gene is not considered to function properly.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Reference will be made to the following documents cited in the international search report:

D1) Protein Science, 4: 1648-1650 (1995), Andersson A et al.
D2) WO02090558 A1
D3) Carcinogenesis, 14(2): 303-305 (1993), Ariza R R et al.

D1 discloses the vector pAA1 comprising the araD gene sequence. The E. coli strain JM109 is transformed with pAA1 in order to overexpress the araD gene and to purify the resulting enzyme, L-ribulose-5-phosphate 4-epimerase (see page 1648, column 2, paragraph 2-3 and the abstract).

Thus, the subject-matter of claim 8 lacks novelty. The fact that the araD gene will function as a selection marker when the vector is inserted in a selection system according to the invention does not confer novelty to the vector as such.

The vector according to dependent claims 9-12 may be novel. However, the construction of expression vectors is well known in the art. D2 discloses an expression vector, which lacks a papilloma virus origin of replication. The vector comprises a DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, and a multimerized DNA sequence forming a binding site for the nuclear anchoring protein. Consequently, it is considered obvious to a person skilled in the art to combine the teachings of D1 and D2 in order to construct vectors having the technical features according to claims 9-12. Therefore, the subject-matter of claims 9-12 is considered to lack an inventive step.

The subject-matter according to claims 13-15 is novel. D3 is considered to represent the closest prior art. D3 describes E. coli strains with a mutation in the araD gene. The inactivation of araD blocks the utilisation of L-arabinose as a carbon source and leads to the accumulation of a toxic intermediate (see page 303, column 2, paragraph 3 and table 1).

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International application No.
PCT/FI 2004/000540

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
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The subject-matter of claims 13-15 differs from what is disclosed in D3 in that other strains of E. coli are mutated in the araD gene. The E. coli strains which are used are the commercially available AG1, JM109 and DH5alpha-T1. It is uncertain what technical effect is achieved due to this difference.

In view of what is known from D3, and in the absence of an unexpected technical effect due to the above mentioned difference, it is considered as an obvious embodiment to the person skilled in the art to construct mutated strains according to claims 13-15. The subject-matter of claims 13-15 is therefore considered to lack an inventive step.

However, claims relating to the use of vectors and mutated strains as described in present claims 8-15 for the construction of a selection system according to the invention would be regarded as novel and inventive.